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Botulinum Toxin as a Therapeutic Agent

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ABSTRACT. Botulinum toxin is a presynaptic neuromuscular blocking agent that, when injected intramuscularly in minute quantities, can produce selective muscle weakness. This property is employed therapeutically to provide symptomatic relief in conditions related to excessive muscle activities in strabismus, blepharospasm, hemifacial spasm, cervical dystonia, spasmodic dysphonia (adductor type), and jaw closing dystonia. It is investigational for a long list of medical conditions. It is a marketed drug in a number of countries in the world, but its use has only been approved by different regulatory agencies for use in a limited number of conditions. The long-term effects, appropriate dose for children, and in pregnancy, and maximum dose without causing toxicity remain unclear. *Copyright © 1996 Elsevier Science Inc.* PHARMACOL. THER. 72(1): 13–24, 1996.

KEY WORDS. Botulinum toxin, serotypes, ophthalmologic, neurologic, dystonia, muscle spasms.

CONTENTS

ABBREVIATIONS. ACh, acetylcholine; BTZ, botulinum toxin; EMG, electromyographic; MU, mouse units.

1. INTRODUCTION

Botulism is a condition related to food poisoning caused by an exotoxin produced by *Clostridium botulinum*. This is a gram-positive, rod-shaped, anaerobic bacterium. In botulism, the affected individual develops signs and symptoms within hours of ingestion of the toxic amounts of contaminated food. The extraocular and bulbar muscles apparently are more rapidly affected, so that the presenting symptoms are usually visual disturbances and bulbar paresis. Generalized muscle weakness may ensue and respiratory paralysis may lead to mortality.

More than seven immunologically distinct antigenic types of botulinum toxins (BTX) have been identified: A, B, C (C1 and C2), D, E, F, and G (Simpson, 1986). Only Types A, B, E, and F have been described to cause human botulism.

In 1973, an ophthalmologist from San Francisco, Dr. Alan Scott, first published a study on the effect of BTX on the lateral rectus muscle of the monkey (Scott *et al.*, 1973). This was performed to search for a therapeutic agent that may produce a temporary muscle weakness in the extraocular muscles, as a possible alternative to surgical treatment of strabismus in children. He included toxic substances such as α-bungarotoxin, disofluorophosphate, ethanol, and BTX type A (BTX-A). BTX-A turned out to be the best agent for this purpose, as it induced transient weakness in the lateral rectus of the monkey, lasting several months without any significant local or systemic side effects. Subsequently, the first application of BTX-A in humans was published in 1981 (Scott, 1981), treating patients with strabismus.

The therapeutic use was then extended to blepharospasm (Scott *et al.*, 1985) and hemifacial spasm (Mauriello *et al.*,

1987; Carruthers and Stubbs, 1987). After its use had been established in cervical dystonia (Tsui *et al.*, 1985, 1986), the commonest form of focal dystonia, neurological applications have been extensive. In recent years, BTX has been investigated in other fields in medicine, such as urology and gastroenterology, and cosmetic applications have been explored.

2. PHARMACOLOGY OF BOTULINUM TOXIN

2.1. *Toxin Structure*

The BTX molecule consists of a heavy chain (H chain, MW 100,000) and a light chain (L chain, MW 50,000). The H chain is important for the binding of the toxin molecule to nerve terminals at the neuromuscular junction. The carboxyl terminal binds to the cell membrane before the L chain is internalized. The latter is the neurotoxic component. The H and L chains are bound together by disulfide bonds, which are heat-labile. The toxin, therefore, can be inactivated by boiling since neither the dissociated H nor the L chain can exert neurotoxicity independently. In one commercial preparation, BTX-A is bound to hemagglutinin and forms a dimer, the complex reaching a molecular weight of 900,000 (DasGupta, 1994).

Different types of BTX may differ in potency by the variation in the amount of proteolytic activities in culture. Proteases cleave a single chain of BTX into two pieces, a process known as "nicking". This will render the toxin much less potent than the unnicked form. Most types are proteolytic, but some strains of Type B and Type E may produce unnicked forms of BTX only.

2.2. *Toxin Action*

BTX acts presynaptically by blocking the release of acetylcholine (ACh) at the neuromuscular junction. It may bind to nerve terminals at autonomic cholinergic ganglia with autonomic effects, but only in very large doses. It is unlikely that therapeutic doses are associated with any significant autonomic adverse reactions. The BTX molecule cannot cross the blood-brain barrier and, therefore, has no CNS effects (Coffield *et al.*, 1994). Three steps are involved in the neurotoxicity: binding, internalization, and neuromuscular blockade.

2.2.1. Binding. Distinct binding sites have been described for different antigenic types of BTX (Black and Dolly, 1986a; Wadsworth *et al.*, 1990). These binding sites have not been clearly characterized.

2.2.2. Internalization. This may involve the amino terminal (N-terminal) of the toxin molecule of the H chain (Lomneth *et al.*, 1993) through an energy-dependent process of endocytosis (Samson, 1984). It has been proposed that an endosome (Black and Dolly, 1986b) is formed around the toxin molecule and provides an acidic pH environment that causes a change in the structure of the molecular (Mellman *et al.*, 1986). This may facilitate a channel for the toxin entry into the cell, the channel being formed of a size just large enough for the L chain to go through (Hoch *et al.*, 1985).

2.2.3. Neuromuscular blockade. The L chain acts as a metalloendopeptidase (Schiavo *et al.*, 1993b) that cleaves the protein complexes necessary for docking of ACh-vesicles to the cell membrane before they can be released. Zinc is an important co-factor (Schiavo *et al.*, 1994). Different types of BTX cleave different parts of the protein complex (Huttner, 1993), as shown Table 1.

2.3. *Pharmacologic Actions*

The action of BTX has been extensively studied *in vitro* using the rat hemidiaphragm model. A series of experiments by Hughes and Whaler (1962) demonstrated that BTX could produce more rapid and more severe weakness in the rat hemidiaphragm when the phrenic nerve was stimulated while BTX was administered. This suggests that the more actively contracting muscles are likely to be taking up BTX more rapidly.

In experiments with mouse phrenic nerve hemidiaphragm, binding to nerve terminal takes about 32-64 min (Yamada *et al.*, 1986). In both animal studies and muscle biopsy obtained from human patients with blepharospasm, muscle atrophy occurs within 2 weeks of injections. This is associated with variation in fibre size. Atrophy continues for about 4 weeks and then stabilizes. It was observed that spreading of the ACh cholinesterase activity occurred to cover most of the toxin-exposed sarcolemma of the muscle. After 4-5 months, this distribution of ACh cholinesterase

TABLE 1. Intracellular Substrates for Different Types of BTXs

BTX serotype	Substrate	Reference
BTX-A	SNAP-25	Schiavo *et al.*, 1993a
BTX-B	VAMP/synaptobrevin	Schiavo *et al.*, 1992
BTX-C	Syntaxin	Blasi *et al.*, 1993
BTX-D	VAMP/synaptobrevin Cellubrevin	Schiavo *et al.*, 1993a
BTX-E	SNAP-25	Schiavo *et al.*, 1993a
BTX-F	VAMP/synaptobrevin Cellubrevin	Schiavo *et al.*, 1993b
BTX-G	unknown	

SNAP-25, synaptosomal-associated protein-25; VAMP, vesicle-associated membrane protein.

activity reverts back to the normal pattern of being confined to the neuromuscular junctions.

Chemical denervation is apparently permanent to the neuromuscular junctions. Recovery takes place by neurogenesis by the formation of axonal sprouts and new motor end plates. Reconnection between nerve terminals and muscle motor end plates takes place. Sprouting occurs within 10 days of exposure (Duchen, 1970).

Functional recovery of the neuromuscular junction takes about 3-6 months, but sprouting and remodeling may continue for up to 3 years (Holds *et al.*, 1990).

2.4. *Quantification*

BTX is quantified in terms of biologic units, the mouse units (MU). One MU is defined as the dose required to kill 50% of a batch of 18–20 g female Swiss–Webster mice (LD_{50}) (Schantz and Kautter, 1978; Schantz and Johnson, 1990).

The LD_{50} for humans, extrapolated from experiments in monkeys, has been estimated to be approximately 40 MU/kg (Botox®) (Scott and Suzuki, 1988). In a 70-kg man, the LD_{50} is, therefore, in the region of 2500-3000 MU. However, species difference in sensitivity to the toxin prevents an accurate calculation of the toxic dose for humans.

2.5. *Preparations*

There are two commercially available BTX preparations. Botox®, marketed by Allergan Inc., Irvine, CA, comes from a batch of toxin prepared by Schantz in 1979 (Schantz and Scott, 1981). In this preparation, 1 ng by weight is equivalent to 2.5 MU. Human serum albumin (0.5%) was added to the crystalline preparation for stability before it is lyophilized. Vials of 100 MU are supplied. The toxin is easily inactivated by heat, shaking, excessive dilution, and surface tension generated from bubbles when the toxin is reconstituted for use.

Dysport® is supplied by Speywood Pharmaceuticals, England, in 500 MU vials. Conflicting reports on the equivalent doses with Botox® have been published. From Poewe's experience on the use of Dysport® in patients with cervical dystonia, an average dose per muscle of 320 MU was employed (Poewe *et al.*, 1992). This suggests that 1 Botox® equals MU 3-5 Dysport® MU.

It is recommended that BTX should be used within 4 hr of reconstitution with normal saline. It has been shown that there is no loss of activity 6 hr after reconstitution at room temperature. However, when left for 12 hr, a loss of up to 44% activity was observed. Refreezing the toxin after reconstitution brings about a 70% loss of bioactivity after 1-2 weeks (Gargland and Hoffman, 1993).

Other types of BTX are investigational. BTX-B has been studied in patients with cervical dystonia (Tsui *et al.*, 1995). Its use will be explored further in patients who are resistant to treatment with type A toxin (Moyer and Setler, 1994). Similarly, the use of BTX-F (Shimizu and Sakaguchi, 1994) remains investigational.

BTX-A is usually diluted with normal saline without preservatives for injections. Dilution of a 100 MU vial, for example, may be performed with 1 mL, 2 mL, 5 mL, or 10 mL of normal saline. This will give a concentration of 10 MU, 5 MU, 2 MU, or 1 MU per 0.1 mL, respectively. It is suggested that lower concentrations are probably easier to use for facial injections, so that the smaller doses are more easily adjusted. For injections using higher doses, more concentrated solutions will enable injections per site to be performed with less volume. There has not been any reported difference in efficacy comparing these different concentrations. However, excessive dilution may lead to some loss in bioactivity of BTX. Tuberculin syringes are used to dilute and draw up the toxin. When injections are performed, new 30-gauge needles are used to reduce discomfort and local trauma/bleeding.

2.6. *Clinical Effects*

When BTX is injected into patients, symptomatic improvement is usually appreciated after a latent period of 1-14 days. The effects peak in about 2-6 weeks, and usually begin to wear off by the end of 10-12 weeks. Muscle atrophy is noted 1-2 weeks after treatment, and the muscle mass usually returns to about 70–80% of the original bulk after 3 months.

3. CLINICAL APPLICATIONS

3.1. *Ophthalmology*

3.1.1. Strabismus. BTX-A was first used experimentally to produce weakness in the lateral rectus of the monkey (Scott *et al.*, 1973). Since 1981, it has been applied to treat patients with strabismus. It works by restoring the balance of antagonistic pairs of extraocular muscles. The injected muscle is made weak and becomes stretched by the unopposed antagonistic muscle, which may result in contracture. When the effects of the toxin wear off, it is hoped that this contracture may perpetuate the correction of the optical axis. It is particularly effective in treating horizontal strabismus (Carruthers and Kennedy, 1991). Results, however, are not as promising in infantile esotropia. Efficacy was only observed in 33% of patients in one series (Biglan *et al.*, 1989). Using electromyographic (EMG) guidance, the injecting teflon-coated needle picks up electrical signals from the extraocular muscle to be injected and BTX is then delivered directly to the target (Carruthers, 1985). This method of therapy has become an established alternative to surgery. It can be performed under topical anaesthesia as an outpatient procedure, but in small children, light ketamine anaesthesia may be required (Scott *et al.*, 1990). Average improvement in angle of deviation was from 80% to 66%, the result being permanent in over half of the cases, but in about 40% of patients, a second set of injections may be necessary.

3.1.2. Entropion. BTX has been used successfully in the treatment of age-related lower eyelid entropion (Carruthers and Stubbs, 1987). Entropion is usually caused by overac-

tivity of certain parts of the orbicularis oculi, and this may result in trichiasis, epiphora, and conjunctival injections. Relief of these symptoms is possible with appropriate injections of BTX into the lower eyelid, as an alternative to surgical correction.

3.1.3. **Other ophthalmologic applications.** BTX-A has been reported to be useful in a variety of ophthalmologic conditions, including vertical strabismus (Magoon and Dakoske, 1985), lateral rectus palsy due to abducens nerve palsy (Rosenbaum *et al.*, 1989), dysthyroid ocular myopathy (Dunn *et al.*, 1986), and nystagmus (Helveston and Pogrebniak, 1988). These applications have to be further evaluated.

3.2. *Neurology*

3.2.1. Dystonia. This is a condition characterized by involuntary muscle contractions in one or many regions of the body. When it occurs in one part of the body, the term focal dystonia is applied. It may involve many parts of the body, in which case, it is called generalized dystonia. The latter can be genetically inherited in an autosomal dominant mode, and a marker has been identified in chromosome 9q in some families (Ozelius *et al.*, 1989). In some, there may be clearly defined inborn errors of metabolism. Neuroleptic drugs may account for some dystonic syndromes. In most cases, the cause is unknown. The commonest form of dystonia, however, is focal dystonia of the neck: cervical dystonia. In the head, it may take the form of blepharospasm (eyelids), oromandibular dystonia (jaws), or Meige syndrome (upper and lower facial dystonia and jaw dystonia). A combination of head and neck dystonia is called cranio-cervical dystonia. In the limbs, it may present as a fixed hand or foot dystonia, or task-specific dystonias. In the latter condition, writer's cramp is commonest, when the patient has a problem writing with the affected hand, but no problem using the hand for any other fine motor tasks. Dystonia may also affect the larynx, producing specific difficulties in phonation. Axial or truncal dystonia may lead to kyphoscoliosis.

3.2.1.1. *Blepharospasm.* The use of BTX has expanded in the field of neurology since it was successfully used in treating blepharospasm (Scott *et al.*, 1985). Blepharospasm is a form of focal dystonia manifested by involuntary contractions of the orbicularis oculi. Patients experience involuntary blinking and closure of the eyelids of varying intensity. They are usually seen by ophthalmologists, who confirm that their eyes are normal. Previous methods of treatment, including oral medications and surgical denervation or myectomy of the orbicularis oculi, have not been satisfactory. Physical means such as crutches attached to custom-made spectacles have been tried and have helped only a small number of patients. BTX injections, starting with 12.5 MU per eye, can bring about symptomatic relief. The dose may be increased to 25 MU per eye or higher, depending on the response. These are usually done in divided doses into 4–5 sites around the orbit. In some cases, the pretarsal fibres may need to be injected to produce a satisfactory result. The middle portion of the upper eyelid is a site to be avoided for fear of ptosis due to inadvertent weakening of the levator palpebrae superiores. The medial portion of the lower lid is also not an injection site since this may lead to tear duct drainage problems and diplopia. EMG guidance is not necessary for this treatment. The orbicularis oculi is a thin muscle that spreads widely around the orbit, particularly after repeated treatment when the muscle would become atrophic. In most instances, s.c. injections of BTX will work just as well.

3.2.1.2. *Cervical dystonia (also known as spasmodic torticollis).* This is characterized by involuntary head and neck movements, either in the form of sustained muscle contractions deviating the head to one side or dynamic jerky intermittent movements. These two types of movements may coexist and cause severe disability. Neck pain with or without headaches are common, occurring in over 70% of patients. Treatment with oral medications give inconsistent results and have not been satisfactory (Lal, 1979). Surgery, including thalamotomy, myelctomy, neurotomy, and selective peripheral denervation of neck muscles (Bertrand *et al.*, 1987), have produced limited benefit, with results varying from surgeon to surgeon. BTX-A injections have become the treatment of choice in recent years, and publications supporting the effectiveness of this treatment are abundant (Tsui *et al.*, 1985, 1986, 1987; Brin *et al.*, 1988; Stell *et al.*, 1988; Gelb *et al.*, 1989; Jankovic and Schwartz, 1990; Jankovic *et al.*, 1990). While it is generally accepted that the treatment is effective in over 80% of cases, with duration of benefit lasting an average of 3-4 months per treatment, controversy exists in certain practical aspects in the methodology of injections. EMG guidance has been advocated by some neurologists (Dubinsky *et al.*, 1991; Comella *et al.*, 1992) to enhance the benefit obtained, but this has not been generally agreed upon. It is likely that in most cases requiring injections into the superficial neck muscles (such as the sternocleidomastoid, splenius capitis, levator scapulae, and trapezius), EMG guidance is not necessary. The dose of BTX has been the focus of controversy for some time. However, the total dose per session is probably less meaningful to consider than the dose per muscle, since the former is dependent on the number of dystonic muscles in the patient. The dose of BTX-A (Botox®) per muscle has been in the region of 50-75 MU according to most publications. Occasionally, doses up to 100 MU per muscle may be used. Injections may be performed in one or two sites per muscle or divided into a number of sites. No significant difference in efficacy has been detected with either method. It is probably practical to divide the doses into 25 MU quanta to be injected in even distribution throughout the length of the muscle. This would be equivalent to 2 injection sites per muscle for 50 MU or 3 sites for 75 MU (Tsui, 1995).

It is now generally agreed upon that "booster" doses should not be given to prevent antibodies from developing in patients, because they may lead to resistance to subsequent treatment.

Benefit is usually observed 2–10 days after a treatment session, and effects would peak in about 4–6 weeks. Duration of effectiveness varies from 10 to 16 weeks, but may vary from 4 weeks to up to 8 months. The average treatment interval is about 3 months. This treatment has been given to patients since 1985, and has remained effective in earliest treated patients for up to 10 years of regular 3-month injections.

3.2.1.3. *Oromandibular dystonia.* This may take the form of jaw closing or jaw opening dystonia. It may also manifest as a persistent jaw deviation to one side. There may be associated dystonia of the face, eyelids, muscles of the pharynx, and the tongue and form part of Meige's syndrome (Meige, 1910). Secondary problems with temporomandibular joints may ensue, and dislocation of the joint may occur due to the dystonic muscular activities. Pharmacotherapy with anticholinergic drugs or benzodiazepines has not been generally rewarding. BTX injections have offered relief in this condition (Blitzer *et al.*, 1989; Hermanowicz and Troung, 1991). Jaw closing dystonia generally responds better to BTX injections than the other varieties. An average of 25–50 MU into each masseter may be effective. This may also be combined with 20–40 MU into each temporalis muscle if results are not satisfactory following injections into the masseters.

Lingual dystonia remains difficult to treat. BTX injections potentially may produce problems of breathing with excessive weakness of the tongue, and should not be performed without close monitoring of the patient.

3.2.1.4. *Laryngeal dystonia.* Production of sound may be impaired by inappropriate contractions of the vocal cords, either with excessive adduction or abduction. The patient has difficulty speaking, resulting in a tight and strained voice, with speech arrest at times in the case of adductor spasmodic dysphonia. A breathy and leaky voice results from abductor spasmodic dysphonia.

Treatment with section of the recurrent laryngeal nerve may provide relief in a small number of patients, but symptoms may recur after a variable period of time. BTX-A injections have been described in various reports to be effective in the adductor variety of dysphonia (Gacek, 1987; Brin *et al.*, 1989). Injections may be performed percutaneously through the cricothyroid membrane into the thyroarytenoid muscles, through a teflon-coated injecting needle guided by EMG monitoring. It remains controversial whether unilateral injections or bilateral injections with smaller doses should be used. In one comparative study, the results are rather similar, reaching over 90% response rate using either method (Ludlow, 1995). In that series, the mean dose required for unilateral injection was in the region of 20 MU (Botox®) and that for bilateral was 5 MU per side. Indirect laryngoscopic approach is a technique preferred by some (Ford *et al.*, 1990). The treatment is also of value in patients who have had recurrent laryngeal nerve section failure or recurrence of symptoms after initial success (Ludlow *et al.*, 1990).

Abductor spasmodic dysphonia is less common and is more difficult to treat. It involves injections of BTX into the posterior cricothyroid muscle (Ludlow *et al.*, 1991), and a dose of 5 MU into each side was successful in improving the voice. Injections may be carried out through the cricothyroid membrane, with the needle going between the arytenoid muscles into the interarytenoid muscle. The toxin will then diffuse into the posterior cricothyroid (Rontal *et al.*, 1991). Another approach is by rotating the larynx and placing the needle behind the thyroid lamina. The posterior cricothyroid muscle will then be accessible (Blitzer *et al.*, 1992). Occasionally, other laryngeal muscles, such as the thyrohyoid or sternohyoid muscles, may need to be injected to achieve better results (Ludlow *et al.*, 1991).

Vocal tremor (Koda and Ludlow, 1992) and adductor laryngeal breathing dystonia (Lew *et al.*, 1992) may benefit from BTX injections.

3.2.1.5. *Limb dystonia.* In the upper limbs, dystonia may manifest as a sustained abnormal posturing of the hand and wrist. However, the commonest form of hand dystonia is writer's cramp, a "task-specific" dystonia. The writing hand quickly develops a distorted posture and pen control is lost. This may or may not be associated with pain in the hand, wrist, forearm, or even up the shoulder. Yet, when the hand is examined neurologically, no abnormality can be detected. Interestingly, if the patient is asked to write on a wall board, writing sometimes can appear normal when more proximal shoulder movements are employed to execute the writing movements. The dystonic hand posture, while writing, can take many different forms: extension, flexion, ulnar or radial deviation, or in various combinations. Different muscles in the forearm and hand are implicated in individual patients. Treatment with conventional methods include oral medications, which are not highly effective, and writing aids (Ranawaya and Lang, 1991), which only help a small number of a selected group of patients. Several open-labeled studies reported effectiveness of BTX-A in the treatment of writer's cramp (Cohen *et al.*, 1989; Rivest *et al.*, 1991). However, these studies probably reflect results of treatment in a highly selected group of patients. In a more recent double-blind study, with sequential recruitment of patients with writer's cramp, it was shown that only 25% of subjects improved significantly in writing after BTX injections (Tsui *et al.*, 1993). A balance has to be struck between relief of dystonic muscle contractions and excessive weakening of functionally important muscles for fine hand movements. Sometimes relief of dystonia may not be possible without too much motor weakness, and treatment with BTX is not practical. In well-selected patients, BTX injections can certainly improve writing significantly. Some dystonic postures when writing are readily helped by this treatment, such as involuntary extension of the index finger, flexion or extension of the wrist, or tight contraction of the long flexors of the fingers, each occurring in isolation. Complex patterns of dystonic posture usually respond poorly to the injections. Other common task-specific

dystonias or occupational cramps include musician's cramp and sportsman's cramp. Treatment with BTX again may be successful in selected groups of patients.

Fixed dystonic posture of the hand usually takes the form of tight finger flexion, wrist flexion with radial or ulnar deviation, and pronation. Other than dystonic contraction of muscles, there is also problems with motor programming in the use of the hand. Relieving the dystonia by BTX injections will improve the posture, but probably not the function of the hand generally. BTX may be given for specific indications, such as relief of pain related to tight muscle contractions, or improving hygienic care of the hand so that it may be opened up more easily for cleaning to prevent infections.

Foot dystonia is probably more worthwhile treating. The dystonic posture in the foot usually consists of plantaflexion and inversion. This will impair walking because the foot cannot be placed flat on the ground, and sometimes there may be difficulty putting on shoes. Injections of 50-150 MU of BTX into the tibialis posterior may relieve foot dystonia for several months and may improve walking.

Dystonias of proximal muscles of upper and lower limbs are more difficult to treat since the muscles are of larger bulk. Movements are more complex, and it requires large doses of BTX if treatment is considered. The modest functional improvement achieved is frequently not sufficient to justify repeated treatment using large doses of BTX, which also makes it not cost effective.

3.2.1.6. *Truncal dystonia*. This may occur in isolation, but is frequently a part of generalized dystonia. Kyphoscoliosis results from dystonic activity of muscles around the vertebral column, as well as abdominal and chest muscles. Treatment with high doses of anticholinergic drugs alone or combined with spasmolytic agents, benzodiazepines, and other medications appears to be effective in 40–50% of cases with generalized dystonia in which truncal dystonia is one component (Fahn, 1995). Intrathecal baclofen infusion may be beneficial, but requires complicated care (Narayan *et al.*, 1991).

BTX injections may offer partial symptomatic relief in patients with truncal dystonia with a major component of extension. Injections of up to 400 MU of BTX into the paraspinal extensor muscles on one side may temporarily relieve dystonia arching backwards and to one side. However, the prevertebral muscles are not accessible to percutaneous injections of BTX, and experience in injecting the abdominal muscles is scanty. Hence, the procedure is not performed in patients with flexion axial dystonia.

3.2.2. Hemifacial spasm. Hemifacial spasm is caused by irritation of the facial nerve, usually at the root exit zone by an anomalous blood vessel, which may be part of the anterior cerebellar, posterior inferior cerebellar, acoustic, or internal auditory arteries. Occasionally, a vein may be implicated. Other causes include recovery from Bell's palsy or structural lesions at the cerebellopontine angle, such as a tumour or an arteriovenous malformation. Anticonvulsants, such as carbamazepine and phenytoin, have offered symptomatic relief in some patients, but these frequently are less effective on long-term use. Surgical microvascular decompression of the facial nerve can effectively cure the condition in most patients (Loeser and Chan, 1983), but serious potential complications, such as facial paralysis, deafness, and stroke, deter many patients from this procedure, in view of the benign nature of the condition.

BTX injections into the facial muscles (Mauriello *et al.*, 1987; Carruthers and Stubbs, 1987; Jankovic *et al.*, 1990) have now become the treatment of choice. This treatment is safe and effective. The technique for injecting the eyelids is the same as in blepharospasm, but other facial muscles may need to be injected. Starting dose for the orbicularis oculi is 12.5 MU (Botox®) and 2.5-5 MU for most other facial muscles. Other commonly injected muscles include the frontalis (5–10 MU), risorius (2.5–5 MU), depressor anguli oris (5 MU), platysma (2.5 MU per strand of muscle that stands out), and zygomatic major (2.5 MU). For the first treatment, one should try injecting the orbicularis oculi alone without treating the lower face, which may improve coincidentally. This may be due to diffusion of the toxin from the upper face influenced by gravity or to the effective stopping of eyelid muscle contractions that may be the "trigger" area for lower facial twitching.

3.2.3. Spasticity. Spasticity is a velocity-dependent resistance to passive movement. It is caused by lesions of the pyramidal system. The involved parts of the body are described as spastic, with associated features of motor weakness (which usually takes up a specific pattern of relative sparing of the physiologically antigravity muscles), increased in muscle tone, hyperreflexia with release of cutaneomuscular reflexes, and impairment of voluntary control. These are also known as "upper motor neuron syndromes".

Any disease of the cerebral cortex, brainstem, or spinal cord may result in spasticity, which is a major cause of disability. Spasticity results in stiffness of joints impairing range of motion, making nursing care and physiotherapy difficult. It may be associated with intermittent spasms that may be painful. Eventually, contractures will develop. Conventional methods of treatment include spasmolytic agents (Rice, 1987; Young and Delwade, 1981a,b), various surgical techniques, including rhizotomy, tenotomy, tendon transfer, and osteotomy, phenol injections (Braun *et al.*, 1973), and intrathecal baclofen (Penn, 1992), have not been satisfactory in a large number of patients. Application of BTX into the spastic muscles is effective in reducing spasticity (Snow *et al.*, 1990). This mode of therapy is now under investigation by many centres in the world, focusing on the following conditions:

3.2.3.1. *Chronic multiple sclerosis*. There are certain situations in which BTX may be considered as a possible therapy. In one instance, patients may be bedridden and afflicted with severe hip adductor spasms. These spasms cause discomfort and pain, and the posture of crossed legs makes

nursing care of the perineum very difficult. In one double-blind study (Snow *et al.*, 1990), injections of BTX-A into the hip adductors (adductors longus, brevis, and magnus) on one side with a total of 400 MU (Botox®) could effectively reduce spasticity and improve the ease of hygienic care. There has not been any report on the repeated use in hip adductor spasm, and the cost effectiveness of long-term treatment is questionable.

BTX may also be considered in situations where patients have developed "spastic ankles". In addition to weakness in dorsiflexion, producing foot drop, they may have spasticity of the ankle plantaflexor, producing an excessively plantaflexed ankle. This impairs walking because they cannot clear the foot off the ground. Even putting a splint on is difficult because of the spasticity with lack of mobility of the ankle. Making the gastrocnemius and the soleus weak increases the flexibility of the ankle and makes putting a splint on easier. Walking can be improved. This application has not been adequately explored.

3.2.3.2. *Post-stroke spasticity*. There has been recent interest in the use of BTX in post-stroke spasticity. Open-labeled studies currently are under way, and the preliminary results appear encouraging (Tsui and O'Brien, 1994). Doses employed were 50-150 MU for bigger muscles, such as the gastrocnemius, soleus, tibialis posterior, tibialis anterior, biceps, brachioradialis, and pectoralis. Two-thirds of 10 patients were pleased with the initial results, but others found no significant functional improvement, despite adequate relief of spasticity. The timing of BTX injections after a stroke has to be considered because treatment has to be given before contractures develop. In general, it is thought that 3-9 months after a stroke would be a good time to enhance physiotherapy and rehabilitation. Further evaluation with double-blind studies is required.

3.2.3.3. *Cerebral palsy*. The application of BTX in cerebral palsy in children has been explored. There has not been any actual dose titration performed in children, but Koman *et al.* (1994) proposed a dose regimen of 6 MU/kg (Botox®). The patients he studied included spastic diplegia, spastic quadriplegia with or without athetosis or with scoliosis. The primary objectives were to reduce spasticity and to improve gait and back posture. His preliminary results suggested that BTX is beneficial in a selected group of patients with cerebral palsy. This application remains investigational at present.

3.2.3.4. *Post-traumatic brain/spinal cord injury*. The use of BTX in peripheral spasticity caused by trauma to the brain or spinal cord should follow the same considerations as those in chronic multiple sclerosis. There is a more specific indication for improving bladder functions in these patients, which will be discussed in Section 3.3.1.

3.2.4. Other neurologic applications.

3.2.4.1. *Tremor*. Head tremor, associated with cervical dystonia or with essential tremor, may be effectively reduced by BTX injections. Bilateral splenius capitis injections are effective in alleviating the horizontal type of head tremor, also described as "no-no" tremor. Sometimes, after treating a patient with cervical dystonia with sustained head deviation with BTX, relaxing the dystonic muscles may unmask an underlying head tremor, and subsequent treatment to this tremor (injecting the splenius capitis on both sides) may be necessary.

In tremor, BTX is helpful in dampening it down (Jankovic and Schwartz, 1991). It is likely to be effective only in well-selected cases. In many patients with essential tremor, it may not be able to reduce tremor significantly without producing severe weakness in the hand. This will lead to functional impairment that offsets the reduction of tremor. In view of the availability of oral medications that are effective for treating essential tremor, the use of BTX should be considered only for those who have disabling tremor and who are resistant to all other forms of therapy.

3.2.4.2. *Tics*. In this condition, patients are suffering from an overpowering impulse to perform repetitive patterns of movements, which may be in different regions of the body. Many of these movements involve the face and neck, and BTX injections have been shown to reduce these movements (Jankovic, 1993). In some patients, the movements may be suppressed after BTX injections, but the impulse to perform the movements may still be present. On the other hand, there are effective oral medications such as tetrabenazine that should remain the first line of therapy.

3.2.4.3. *Stuttering*. In stuttering, there is an impairment of control of speech, so that sentences may be broken and words get repeated. The actual mechanism of this remains unknown. EMG studies, however, demonstrated that increased muscular activities are associated with speech blocks (Kolotkin *et al.*, 1979). Spontaneous bursts of muscle activities are also found in stutterers when they are not making any sounds (Schapiro, 1980). There are also muscle overactivities in the face and jaw, and these together form the rationale for using BTX as a method of treatment. The results of an open-labeled study were encouraging (Ludlow, 1990). Further studies are required to evaluate the efficacy of BTX in stuttering.

3.2.4.4. *Palatal myoclonus*. This is usually caused by lesions at the inferior olivary nucleus, with astrocytic proliferation leading to a macroscopic appearance of hypertrophy of the nucleus (Gautier and Blackwood, 1961). The major symptom is clicking in the ear, due to contraction of the levator veli palatini or tensor veli palatini muscle, though others suggest contraction of the stapedius. Treatment has been difficult. Medications, such as clonazepam or 5-hydroxytryptophan, have been reported to be effective only in some patients. BTX injections into the palatini muscles have been reported to be successful in controlling the symptoms (Deuschl *et al.*, 1991). Regurgitation of fluids could take place due to weakness of the soft palate after injection. This condition is uncommon, and reports on the use of BTX remain scanty.

3.3. *Miscellaneous Applications*

3.3.1. Detrusor-sphincter dyssynergia. Bladder control is commonly affected after spinal cord injury. Spasms of the detrusor-sphincter lead to building up of high pressure in the bladder, with poor emptying. This, in turn, leads to complications such as urinary tract infection and back pressure damage to the kidneys. Management methods include oral medications, condoms, and indwelling catheters. These are prone to long-term complications. BTX injections into the striated muscle sphincter of the bladder have been proposed to be an alternative to conventional therapy (Dykstra *et al.*, 1988). This subsequently was followed by a double-blind study that confirmed the results (Dykstra and Sidi, 1990). The injections are performed via a cystoscope using a teflon-coated needle that can record EMG activities. Several injections may be required to produce adequate reduction in residual urine volume. These encouraging results should be followed by more evaluation studies, and dose finding is certainly necessary.

3.3.2. Anismus. This is a condition of chronic constipation associated with spontaneously increased tone of the external anal sphincter (anismus) (Preston and Lennard-Jones, 1985). In some patients, symptoms may be so disturbing that posterior division of the puborectalis muscle has been performed as a surgical procedure for relief. BTX, injected into the external anal sphincter, has been shown in a double-blind study to be able to relieve spasm of the sphincter, reduction of intrarectal pressure, with improvement in constipation (Hallan *et al.*, 1988). Treatment of anal fissures by injecting the internal anal sphincter has also been proposed.

3.3.3. Achalasia. Based on experiments demonstrating activity of BTX in the myenteric plexus (Bigalke and Habermann, 1980), this property has been employed to treat gastrointestinal disorders associated with hyperactivity of sphincter muscles. Achalasia is associated with increased contractions of the lower oesophageal sphincter, and with reduced or absent peristalsis. Nitroglycerin or calcium channel blockers have been employed to relax the smooth muscles at the lower oesophageal sphincter, but results have been variable. Myotomy of the sphincter may relieve symptoms of regurgitation, but carries a high incidence of gastro-oesophageal reflex. Pneumatic dilatation is not as effective and may cause oesophageal perforation. BTX injections, therefore, become an attractive alternative. BTX was successful in a patient who failed with conventional methods of therapy (Pasricha *et al.*, 1993b). This same group has also reported encouraging results treating dysfunction of the sphincter of Oddi (Pasricha *et al.*, 1993a).

3.3.4. Cosmetic. Treatment of glabellar frown lines and other facial wrinkles with BTX-A have been reported to be successful (Carruthers and Carruthers, 1992, 1994). This is based on the principle that facial wrinkles or frown lines are produced by overactivity of facial muscles. The wrinkles are usually perpendicular to the line of contraction of the facial muscles. Treatment needs to be repeated once every 3–6 months in most cases.

4. ADVERSE REACTIONS

No serious adverse reactions have been reported from the use of BTX injections. More serious potential complications are related to poor technique of injections. Side effects due to BTX may occur due to excessive weakening of the injected muscles or diffusion of the toxin to the neighbouring muscles. When they occur, the side effects are always transient and invariably resolve spontaneously within weeks.

Complications related to poor technique include eyeball injury and pneumothorax, depending on the site of the injections. It is important, therefore, that the treating physician know the regional anatomy well before such a procedure is undertaken.

4.1. *Systemic Effects*

These include fever, malaise, fatiguability, and flu-like symptoms. However, in one double-blind study, these systemic effects occurred more commonly after placebo injections than after BTX injections (Tsui *et al.*, 1986).

Single-fibre EMG changes have been reported to be detected in muscles remote from the site of injections (Sanders *et al.*, 1986), implying distant systemic effects from BTX. The significance of this finding is unclear. There is, however, no clinical motor weakness detected in muscles away from the site of injections.

4.2. *Excessive Weakness in Target Muscles*

Neck weakness may occur in patients with cervical dystonia. Occasionally, the patient may have difficulty keeping the head up, particularly after injections into the posterior neck muscles for retrocollis. Excessive weakness of muscles of the tongue may lead to respiratory embarrassment. Vocal cord injections may result in a breathy voice for the adductor variety, and respiratory problems for the abductor variety. Too much weakness induced in the hand muscles may lead to transient functional impairment.

4.3. *Local Complications and Diffusion*

Injections of BTX into different parts of the body may produce local complications either by inadvertent injury of tissues or structures or by local diffusion of the toxin into neighbouring muscles producing undesirable weakness. Ecchymosis is sometimes inevitable, especially around the orbit.

When used in the treatment of strabismus, the incidence of complications remain low. Perforation of the eye or periocular hemorrhage may occur at a frequency of less than 1%, and all reported cases have responded to appropriate treatment, with restoration of normal vision (Carruthers and Kennedy, 1991). Partial ptosis or secondary vertical deviations may result, but these are usually transient, self-limiting, and do not lead to amblyopia.

For facial injections, ptosis can occur when the orbicularis oculi is injected, due to diffusion of BTX to the levator palpebrae superiors. This complication may be prevented by avoiding the middle portion of the upper eyelid. Tear drainage problems and diplopia may be prevented by avoiding the medial portion of the lower eyelid. Droopiness of the angle of the mouth may occur. Vocal cord injections may result in a breathy voice in the treatment of adductor dysphonia.

Dysphagia is a complication that is related to diffusion of BTX to the pharyngeal muscles. Frequency of occurrence ranges from 1.7% (Tsui *et al.*, 1987) to 90% (Stell *et al.*, 1988), depending on the centre performing these injections. It has been suggested to be more common with bilateral sternomastoid injections (Comella *et al.*, 1992) or related to the dose injected into the sternomastoid muscle (Borodic *et al.*, 1990), but these findings have not been consistent.

4.4. *Immunologic*

There has not been any truly documented case of hypersensitivity reactions to BTX injections. Antibody formation is probably of most concern since this may render further injections of BTX ineffective. Resistance to BTX injections may occur, frequency varying in different centres, probably ranging from 0% to over 25% (Tsui *et al.*, 1988; Zuber *et al.*, 1993). Antibody formation is probably dose dependent, since it has been reported in patients with cervical dystonia receiving doses in 150–300 MU range, but has not been observed in patients receiving doses of up to 52.5 MU per session for facial spasms for up to 3 years (Gonnering, 1988). It is probably also influenced by the frequency of treatment sessions, when "booster" doses given within a short time may play a role in enhancing antibody formation. Inadvertent injection of BTX into the systemic circulation may also have a similar effect.

It is difficult to carry out prospective studies on the factors enhancing antibody formation because the currently available antibody assay technique is expensive, cumbersome, and is not generally available. This is a neutralizing antibody assay using a group of mice to observe the protective effects from the patient's serum on the BTX-inoculated mice (Hatheway *et al.*, 1984). Serial dilution of the patient's serum gives the titre semiquantitatively. Currently, newer methods of assay are being developed, employing enzyme-linked immunosorbent techniques (Tsui *et al.*, 1986; Dezfulian *et al.*, 1984). However, these methods have not been validated.

5. LIMITATIONS AND OTHER CONSIDERATIONS

It should be stressed that BTX is a symptomatic treatment and is not a cure for the conditions it is used to treat. In most cases, injections are carried out repeatedly at regular intervals of 3–4 months. The maximal dose without toxicity is unclear. It is generally agreed that total doses of less than 400 MU may be given per treatment session without any significant side effects. With a certain limitation on the total dose, it is understandable that BTX can only be given to a limited number of muscles at any treatment session. More proximal limb muscles and truncal muscles require significantly larger doses than the distal limb, neck, and facial muscles. It takes, for instance, 400 MU to significantly reduce hip adductor spasm on one side (Snow *et al.*, 1990) in chronic multiple sclerosis. Therefore, it is difficult to reduce spasticity in other regions of the body in addition, without going to toxic doses of BTX.

For hand dystonia, it frequently is not possible to reduce dystonic activities without producing significant weakness in the muscles injected. The weakness induced may lead to functional impairment, which may offset the benefit obtained by reduction of dystonic contractions. Such is particularly true for writer's cramp, where improvement in writing may be associated with deterioration in other fine motor tasks that were otherwise normal before treatment.

In conditions such as chronic multiple sclerosis, cerebral palsy, and stroke, spasticity is also associated with significant muscle weakness. Reducing spasticity does not necessarily improve the functions of these patients. Care must be exercised in selecting the appropriate patients for BTX injections.

Caution should be exercised when using BTX in children and in pregnancy. No definite adverse effects have been reported in pregnancy, but treatment should be avoided as far as possible. In neuromuscular disorders, such as amyotrophic lateral sclerosis, myasthenia gravis, and Eaton-Lambert syndrome, BTX should be used only after careful consideration, and with smaller doses to start off with.

6. CONCLUSIONS

The impact of the use of BTX in the treatment of focal dystonias has been paralleled to the discovery of levodopa in alleviating parkinsonian symptoms. The therapeutic applications have been extended to many fields in medicine. It is clear that BTX can relieve many forms of involuntary and persistent muscle contractions. In many instances, improvement in posture and appearance can be achieved. However, it is important to consider how much functional improvement one can bring about. Each patient needs to be considered separately, and the indication and objective of treatment should be made very clear.

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